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Aerosols Containing *Legionella pneumophila* Generated by Shower Heads and Hot-Water Faucets

GARY E. BOLLIN,[1,2]* JOSEPH F. PLOUFFE,[2] MICHAEL F. PARA,[2] AND BARBARA HACKMAN[2]

Youngstown Hospital Association, Youngstown, Ohio 44501,[1] and Department of Medicine, Division of Infectious Diseases, The Ohio State University Medical Center, Columbus, Ohio 43210[2]

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Shower heads and hot-water faucets containing *Legionella pneumophila* were evaluated for aerosolization of the organism with a multistage cascade impaction air sampler. Air was collected above two shower doors and from the same rooms approximately 3 ft (91 cm) from the shower doors while the hot water was running. Low numbers (3 to 5 CFU/15 ft^3 [0.43 m^3] of air) of *L. pneumophila* were recovered above both shower doors, but none was recovered from the air in either room outside the shower door. Approximately 90% (7 of 8 CFU) of the *L. pneumophila* recovered were trapped in aerosol particles between 1 and 5 μm in diameter. Air was collected 1 to 3 ft (30 to 91 cm) from 14 sinks while the hot water was running. Low numbers (1 to 5 CFU/15 ft^3 of air) were recovered from 6 of 19 air samples obtained. Approximately 50% (6 of 13 CFU) of the organisms recovered were trapped in aerosol particles between 1 and 8 μm in diameter. Shower heads and hot-water taps containing *L. pneumophila* can aerosolize low numbers of the organism during routine use. The aerosol particle size is small enough to penetrate to the lower human respiratory system. Thus, these sites may be implicated as a means of transmission of *L. pneumophila* from potable water to the patient.

The acquisition of nosocomial Legionnaires disease has been linked to inhalation of aerosols containing *Legionella pneumophila*, originating from cooling towers (9), humidifiers (24), soil excavation sites (21), and respiratory therapy equipment (2). Outbreaks have also been reported in hospitals, where *L. pneumophila* was recovered from shower heads and potable water (8, 12, 19, 20, 22). Inhalation of aerosols generated by showers or taps is assumed to be the mode of transmission (3, 15) in these outbreaks. An experimental form of pneumonic Legionnaires disease has been produced in guinea pigs by exposing them to aerosols of concentrated potable water from a hospital where nosocomial Legionnaires disease had occurred (14). Previous investigations, however, have failed to demonstrate *L. pneumophila* in aerosols generated by contaminated shower heads from hospitals where nosocomial Legionnaires disease has been observed (11; A. H. Woo, V. L. Yu, and A. Goetz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, L22, p. 382).

Plouffe et al. (17) described nosocomial Legionnaires disease associated with recovery of *L. pneumophila* from potable water. To define better the mode of transmission from these sites to the patient, we tested shower heads and hot-water faucets in patient rooms for aerosolization of *L. pneumophila*.

MATERIALS AND METHODS

Environmental sampling sites. Cultures of potable water from taps and shower heads were performed as described by Plouffe et al. (17). Hot-water taps and shower heads were turned on, and the initial 10 ml of water was collected. The faucet was turned off, and the inside of the shower head or tap was swabbed with a sterile, cotton-tipped swab before an additional 40 ml was collected. The swab was rinsed in the collection tube and removed. All water culture data in this report represent a combination of the organisms in the water and those rinsed off the swab. The sample was centrifuged at 1,000 × *g* for 30 min, the supernatant was poured off, and the sediment (0.5 ml) was suspended in 4.5 ml of 0.2 N HCl-KCl solution (pH 2.5) for 10 min (6). Portions of 0.1 ml of the acid-treated sample were plated onto buffered charcoal-yeast extract (BCYE) agar (Remel, Lenexa, Kans.).

Voss et al. (23) had previously reported that most of the organisms recovered from potable water came from the initial volume sampled, which represented the stagnant water in the pipe between the valve and the tap or shower head. We did not want to alter the typical situation by removing the water that held most of the organisms before sampling the air, so water cultures were obtained 1 week before air sampling, but not on the day of sampling.

Air sampling devices. An Andersen 1 AFCM viable (microbial) particle sizing sampler (Andersen Samplers, Inc., Atlanta, Ga.) was used during the initial phase of the investigation. This apparatus consisted of six round aluminum stages clamped together to form a sealed cylinder, with an air inlet above the top stage and a vacuum connection below the bottom stage. Each stage held a removable glass petri dish holding BCYE agar without antibiotics (made in our laboratory by the method of Edelstein [10]) and had air vents above the agar surface, which became progressively smaller as stages progressed from top (1.81 mm, stage 1) to bottom (0.25 mm, stage 6). These vents functioned as air jets when air was drawn through the sampler and forced suspended particles of progressively smaller size (≥7 μm at the top; 0.65 to 1.1 μm at the bottom) onto the agar at each stage. This arrangement approximated penetration of the particles into progressively lower levels of the human respiratory system, with stages 4, 5, and 6 representing penetration to secondary bronchi (2.1 to 3.3 μm), terminal bronchioles (1.1 to 2.1 μm), and alveoli (0.65 to 1.1 μm), respectively (1). The accuracy of the sampling process was dependent on a constant airflow of 1 ft^3/min (28,315 cm^3/min) through the system, provided by a continuous-duty vacuum pump attached to the bottom stage. Flow was calibrated by a dry gas meter (1).

An Andersen 1 AFCM two-stage viable particle sampler

* Corresponding author.

(Andersen Samplers, Inc.) was used in later phases of the investigation. This sampler only had two aluminum stages with different sizes of air vents, which enabled differentiation of nonrespirable (≥8 μm, stage 1) from respirable (0.8 to 8 μm, stage 2) particles and allowed use of commercially prepared BCYE agar in disposable plastic plates.

Air sampling in showers. The six-stage sampler was used to evaluate two shower rooms as follows. The sampler was placed on a ledge above the shower door, out of reach of the spray from the shower head. The hot water and air sampler were turned on simultaneously and allowed to run for 15 min; then both were turned off. The procedure was repeated for a second 15-min period after removing the culture plates and replacing them with fresh plates. The hot water was turned on normal force for the shower sampling periods and for tap sampling periods described below to simulate conditions of patient exposure.

The two-stage sampler was used at a later date and in a different fashion. The sampler was placed on a platform in the room 3 ft (91 cm) from the shower door and 2 ft (61 cm) above the floor to avoid splashing. The air was sampled for 10 min before the hot water was turned on. The culture plates were removed and replaced with fresh plates, and then the hot water and sampler were turned on simultaneously and allowed to run for 10 min. The water and sampler were turned off, the culture plates were again replaced, and the air was sampled for 10 min after the water was turned off.

Air sampling in sink areas. Air samples were obtained from 14 rooms. A total of three rooms were tested with the six-stage sampler. The sampler was placed 2 ft from the tap and turned on simultaneously with the hot water. Air was sampled continuously for 15 min; then both the sampler and the hot water were turned off.

The remaining 11 rooms were tested with the two-stage device. Of these rooms, three were tested twice, on different dates. One room was tested three times. The sampler was placed 1 to 3 ft from the tap. Air was sampled for 5 to 15 (usually 15) min before the water was turned on, and then the culture plates were replaced. The hot water and sampler were turned on simultaneously and allowed to run for 5 to 15 min. The culture plates were replaced, and the air was sampled for an additional 5 to 15 min after the water was turned off. The sampling periods before and during the time when the water was turned on and after it was turned off were the same for a given sink.

Handling of cultures. Culture plates were incubated in room air at 35°C and initially examined at 72 h after removal from the sampler. Plates were further incubated for up to 7 days to look for slow-growing organisms. Suspicious colonies were subcultured onto BCYE agar and sheep blood agar (GIBCO Diagnostics, Madison, Wis.). Colonies growing on BCYE agar but not on sheep blood agar were tentatively identified as *L. pneumophila*. Identification was confirmed by direct fluorescent antibody stain (7) with *L. pneumophila* group 1 fluorescein isothiocyanate-labeled rabbit globulin (Biological Products Division, Centers for Disease Control, Atlanta, Ga.). Subtyping of isolates was performed by microagglutination with hybridoma media containing monoclonal antibody LP-I-81 (16).

RESULTS

Air sampling in showers. A total of two paired water and air samples were obtained from each of the two shower rooms. All four water cultures grew *L. pneumophila*. Low numbers of aerosolized *L. pneumophila* (3 to 5 CFU/15 ft^3 [0.43 m^3] of air) were recovered when the air was sampled above the shower doors with the six-stage sampler (Table 1). Equal numbers of organisms were recovered in the first and second 15-min sampling periods. Of the 8 CFU recovered, 7 grew on plates from stage 3 or lower. No *L. pneumophila* was recovered when the two-stage sampler was placed outside the shower door and closer to the floor.

TABLE 1. Rooms with positive air cultures[a]

Room	Water culture[b] (CFU/ml)	Air culture			Sampler penetration	
		CFU	ft^3 sampled	CFU/ft^3	CFU	Stage
3E[c]	>200	5	30	0.17	1	1
					1	3
					2	4
					1	6
5W[c]	>200	3	30	0.10	1	3
					2	5
Sinks:						
188[c]	>200	2	15	0.13	1	1
					1	3
514[d]	3	3	15	0.20	3	2
518[d]	200	5	15	0.33	5	1
557[d]	187	1	15	0.07	1	2
948[d]	23	1	5	0.20	1	1
1158[d]	0	1	10	0.10	1	2

[a] Data are shown only for rooms with positive aerosol cultures. Rooms from which no *L. pneumophila* were recovered by air sampling were omitted.
[b] Water culture results reflect organisms recovered from both water and swabs obtained 1 week before air sampling.
[c] Six-stage sampler used.
[d] Two-stage sampler used.

Air sampling in sink areas. A total of 19 paired water and air samples were obtained from 14 hot-water faucets. A total of 17 of the water cultures grew *L. pneumophila*. Two colonies of *L. pneumophila* (one on stage 1, one on stage 3) were recovered from air around one of the three taps tested with the six-stage unit (Table 1). A total of 11 colonies were recovered (6 on stage 1, 5 on stage 2) from 5 of the remaining 13 taps tested with the two-stage unit (Table 1). All positive air cultures from the two-stage unit were obtained during the period when the tap water was running. None were ever obtained before the tap water was turned on or after it was turned off. No air cultures were positive more than once among the rooms that were tested two and three times. Organisms recovered from the water cultures and air samples from a given tap always gave the same microagglutination with LP-I-81.

DISCUSSION

Previous investigations have failed to demonstrate aerosolization of *L. pneumophila* from contaminated shower heads. A multicenter study of nosocomial Legionnaires disease associated with isolation of *L. pneumophila* from hospital shower heads was unable to prove the hypothesis that the aerosol generated by a shower head was the mode of transmission (8). Hanrahan et al. (11) reported that patients with nosocomial Legionnaires disease were significantly closer than controls to showers contaminated with *L. pneumophila* but were unable to isolate it from aerosols around the showers by using air monitoring equipment. Woo et al. recovered *L. pneumophila* from aerosols produced by a contaminated humidifier but could not detect the organism by settle plates or with an air aspirator when contaminated

showers were tested (Woo et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1985).

We recovered *L. pneumophila* when our sampler was placed in the path through which the steam exited from the shower stall, but not when it was placed in the room only a short distance (3 ft) from the shower. Most (approximately 90%) of the aerosolized *L. pneumophila* was trapped in particles small enough to reach the lower respiratory system as determined by penetration into the lower levels of the sampling chamber (stages 3 to 6 in the six-stage sampler and stage 2 in the two-stage sampler).

Our observations may explain why showering is a risk factor in some cases of nosocomial Legionnaires disease. The data cannot be extrapolated to explain nosocomial Legionnaires disease in patients who were in rooms closer to shower areas than were controls (11) because our cultures were negative a short distance from the showers. Our study was not designed specifically to address the epidemiologic link between showering and acquisition of nosocomial Legionnaires disease. We only wished to determine if shower heads and taps could generate aerosols containing viable *L. pneumophila*.

Little or no data exist regarding aerosol production from hot-water taps. Our investigations show that hot-water taps can produce aerosols containing *L. pneumophila*. Taps may be less efficient aerosolizers than shower heads since we recovered *L. pneumophila* from only 6 of 19 taps as compared with two of four shower heads, although the number of samples is too small for statistical analysis. The organisms recovered from the air around taps were equally distributed between the plates containing the respirable particles (≤ 8 μm) and those containing the nonrespirable particles. Most of the *L. pneumophila* recovered from the air around shower heads, however, were deposited on plates from stage 3 (≤ 5 μm) or lower. This difference suggests larger particle generation by the taps. On two occasions, air sample cultures were positive when water cultures from the previous week grew zero and three colonies of *L. pneumophila*, suggesting that heavy contamination at the tap may not be required to produce contaminated aerosols. The recovery of organisms from the aerosols when prior water cultures were negative, however, can more likely be explained by fluctuation in tap water contamination. We have observed weekly fluctuations from 0 to >200 CFU of organisms recovered from the water. We did not attempt to correlate the number of organisms in the water with the number in aerosols, for the reasons given in Materials and Methods.

Experimentally administered aerosols containing <129 CFU of *L. pneumophila* have produced nonlethal infection in guinea pigs (5). Although no one knows the minimum infectious dose for humans, the low numbers of organisms recovered from the aerosols in our study seem small relative to the expected organism load required to produce human disease. It is possible that low relative humidity at the time of the sampling decreased the number of viable organisms recovered, as had been reported previously (4). Another possible reason for low bacterial counts is the mechanical trauma and dehydration the organisms sustain during the sampling procedure, caused by forcing them through small orifices and onto the agar surface at high speed (13). It is also possible that the numbers of organisms present in shower or tap water were too low to generate large numbers of aerosolized organisms.

Our data show that shower heads and sinks can produce fine particle aerosols containing low numbers of *L. pneumophila* during routine use. The aerosol size is small enough to penetrate to the lower respiratory system. Organisms in larger particles recovered from stage 1 of both samplers, although too large to penetrate to the lower respiratory tract, may be capable of colonizing the oropharynx (18). This study lends supportive evidence to the widely held presumption that aerosols from shower heads and hot-water taps can be implicated as a means of transmission of *L. pneumophila* from potable water to the patient. The acquisition of Legionnaires disease by patients a distance from the site of aerosol generation, however, cannot be explained by our data.

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